SECOND SUPPLEMENTAL DECLARATION

EXHIBIT E

PUBLICATIONS (3)

Studies on the Average Content of Nucleic Acids in Human Marrow Cells. By J. N. DAVIDSON, I. LESLIE and J. C. WHITE. (*From the Department of Biochemistry, University of Glasgow, and the Department of Pathology, Postgraduate Medical School of London*)

In extension of previously reported analyses of the deoxyribonucleic acid phosphorus (DNAP) and ribonucleic acid phosphorus (RNAP) content of aspirated human bone marrow (Davidson, Leslie & White, 1947, 1948), we now report a modification involving enumeration of the nucleated cell content of the samples analysed. Results are expressed in terms of DNAP and RNAP per cell (Table 1), and are average values for the growing and adult cell populations of the analysed samples. The recent results of Vendrely & Vendrely (1948, 1949) and of Mirsky & Ris (1949) suggest a striking constancy in the DNAP content of normal cell nuclei from the tissues of any given species, and our figures for DNAP are of the same order as those quoted by the Vendrelys for human liver nuclei.

There is no significant difference between the means for the normal and the leukaemic series, either as a whole, or considering only acute leukaemia prior to therapy.

A small series of 6 cases of iron-deficiency anaemia has not shown significant variation of the mean DNAP and RNAP per cell from normal.

Results obtained from cases of pernicious and other megaloblastic anaemias are shown in Tables 2 and 3.

It must be noted clearly that the group under therapy cannot be considered as returned to normal, either as regards blood picture, marrow cytology or adequacy of therapy. The significant fall in RNAP from that in the group prior to therapy parallels the general increase in maturity of the marrow under therapy. Cases fully treated and returned to normal are under investigation.

Table 1

Normal human marrow

Values of Nucleic Acid Phosphorus (NAP) in $\mu g. \times 10^{-7}$ per cell

	DNAP 18 obs. on 16 individuals	RNAP 20 obs. on 18 individuals	Ratio RNAP/DNAP
Mean	8·54	6·33	0·75
S.E. of obs.	2·89	3·03	0·326
Observed range	4·0–15·0	2·1–13·5	0·43–1·9

Marrow from cases of leukaemia of various types, before and during therapy

	28 obs. on 15 cases	24 obs. on 12 cases	
Mean	8·75	7·59	0·90
S.E. of obs.	3·05	3·72	0·30
Observed range	3·9–17·4	2·6–17·4	0·3–1·8

Table 2. *Cases of pernicious anaemia and other megaloblastic anaemias*

NAP in $\mu g. \times 10^{-7}$ per cell

		DNAP	RNAP	Ratio DNAP/RNAP
Group as a whole		28 obs. on 12 cases		28 obs. on 13 cases
	Mean	12·6	10·9	0·87
	S.E.	4·56	5·03	0·27
	Observed range	6·6–22·8	2·3–25·1	0·35–1·5
Group prior to therapy		12 obs. on 12 cases	11 obs. on 11 cases	12 obs. on 12 cases
	Mean	12·57	13·38	1·06
	S.E.	4·17	5·19	0·249
	Observed range	8·1–22·8	7·5–25·1	0·69–1·5
Group during the course of therapy		17 obs. on 8 cases	15 obs. on 8 cases	16 obs. on 9 cases
	Mean	12·63	9·09	0·73
	S.E.	4·36	4·21	0·198
	Observed range	6·6–18·8	2·3–17·6	0·35–1·0

Table 3. t *test of significance between means*

		DNAP	RNAP	Ratio RNAP/DNAP
Megaloblastic series as a whole compared with normal series	*P*	<0·001	<0·001	0·2–0·1
	Degrees of freedom	44 Highly significant	44 Highly significant	46 Not significant
Megaloblastic series before therapy compared with normal	*P*	0·01–0·001	<0·001	0·01–0·001
	Degrees of freedom	28 Highly significant	29 Highly significant	30 Highly significant
Megaloblastic series during therapy compared with normal	*P*	0·01–0·001	0·05–0·02	0·8–0·7
	Degrees of freedom	33 Highly significant	33 Significant	34 Not significant
Megaloblastic series before and during therapy compared	*P*	0·7–0·6	0·05–0·02	<0·001
	Degrees of freedom	27 Not significant	24 Significant	26 Highly significant

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Fluoroacetate Poisoning and 'Jamming' of the Tricarboxylic Acid Cycle; Mode of Action of an 'Active' Fluoro Compound Synthesized via this Cycle.

By P. Buffa, W. D. Lotspeich, R. A. Peters and R. W. Wakelin. (*Department of Biochemistry, University of Oxford*)

So far no isolated enzyme has been inhibited by fluoroacetate. The hypothesis has been advanced by Liébecq & Peters (1949) (see also Martius, 1949) that the inhibition of citrate oxidation, occurring also *in vivo* (Buffa & Peters, 1949), is due to the 'jamming' effect of an enzymically synthesized fluorotricarboxylic acid in the Krebs tricarboxylic acid cycle. In support of this hypothesis, Buffa, Peters & Wakelin (1950) have isolated, from guinea-pig kidney homogenates treated with fluoroacetate, a tricarboxylic fraction, which is 'active' in preventing disappearance of added citrate. This active fraction is mainly citrate; it contains no fluoroacetate, but there is present a small amount of a F-compound which is chromatographically inseparable from the tricarboxylic acids.

We have tried to find the exact point of inhibition in the enzymes of the tricarboxylic acid cycle by determining the effect of the 'active' fractions upon aconitase (Johnson, 1939), isocitric dehydrogenase (Adler, Euler, Günther & Plass, 1939) and oxalosuccinic decarboxylase (Ochoa & Weiss-Tabori, 1948), obtained from rat and pig heart tissue. Tables 1, 2 and 3 show that the results were negative, even when amounts of 'active' fraction were used 80 times larger than those inhibiting citrate disappearance in the kidney homogenates.

All the evidence from experiments *in vivo* and *in vitro* (? mitochondrial homogenates) points to inhibition by the 'active' compound at either the aconitase or isocitric dehydrogenase stage. Hence, we are led to the conclusion that the complete system has properties not present in its isolated enzyme components. Whether these be due to factors of organization or to missing components must be decided by further work.

Table 1. *Rat heart aconitase*

	Citric acid (μmol.)	
Time (min.) ...	0	60
Additions:		
cis-Aconitate (5 μmol.)	0·21	3·90
cis-Aconitate + 'active' fraction	0·08	3·96
Citrate (5 μmol.)	4·90	4·34
Citrate + 'active' fraction	5·27	4·38

Table 2. *Pig heart isocitric dehydrogenase*

	$E_{340\,m\mu}$ (max. value)
DL-*iso*citrate only	0·076–0·065
Same + 'active' fraction	0·075
Same + *p*-chloromercuribenzoic acid $1·33 \times 10^{-4}$ M	0·004

Table 3. *Pig heart oxalosuccinic decarboxylase*

(CO_2 evolution from 10 μmol. oxalosuccinate in 30 min. at 13·5° C. Net values)

	CO_2 (μl.)
Enzyme alone	83
Enzyme + 'active' fraction	76
Enzyme + DL-*iso*citrate (control)	14

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Nucleic Acids

Content and Distribution

Nucleic acids in an average human cell

DNA	~6 pg/cell[a]
Coding sequences	3% of genomic DNA
Number of genes	0.51.0 x 10^5
Active genes	1.5 x 104
Total RNA	~10 50 pg/cell[b]
rRNAs	80 85% of total RNA
tRNAs, snRNAs, and low mol. wt. RNA	15 20% of total RNA
mRNAs	1 5% of total RNA
nuclear RNA	~14% of total RNA
Ratio of DNA:RNA in nucleus	~ 2:1
Number of mRNA molecules[c]	0.2 1.0 x 10^6
Number of different mRNA species	1.0 3.4 x 10^4
Low abundance mRNA (5 15 copies/cell)	11,000 different messages
Intermediate abundance mRNA (200 400 copies/cell)	500 different messages
High abundance mRNA (12,000 copies/cell)	<10 different messages
Abundance of each message for:	
Low abundance mRNA (5 15 copies/cell)	<0.004% of total mRNA
Intermediate abundance mRNA (200 400 copies/cell)	<0.1% of total mRNA
High abundance mRNA (12,000 copies/cell)	3% of total mRNA

a 30 – 60 µg/ml blood for human leukocytes.
b 1 – 5 µg/ml blood for human leukocytes.
c Average size of mRNA molecule = 1930 bases.

RNA content of cells in culture

Type of cell	Total RNA (mRNA (µg/107 cells)	mRNA (µg/107 cells)
NIH/3T3 cells	75 200	1.5 4.0
HeLa cells	100 300	2 6
CHO cells	200 400	3 6

Table of Content

UMRECHNUNGSTABELLEN

I. Conversiontable

Molecular weight (daltons)	1µg	1nmole
100	10 nmoles or 6 x 10^{15} molecules	0.1 µg
1,000	1 nmole or 6 x 10^{14} molecules	1 µg
10,000	100 pmoles or 6 x 10^{13} molecules	10 µg
20,000	50 pmoles or 3 x 10^{13} molecules	20 µg
30,000	33 pmoles or 2 x 10^{13} molecules	30 µg
40,000	25 pmoles or 1.5 x 10^{13} molecules	40 µg
50,000	20 pmoles or 1.2 x 10^{13} molecules	50 µg
60,000	17 pmoles or 10^{13} molecules	60 µg
70,000	14 pmoles or 8.6 x 10^{12} molecules	70 µg
80,000	12 pmoles or 7.5 x 10^{12} molecules	80 µg
90,000	11 pmoles or 6.6 x 10^{12} molecules	90 µg
100,000	10 pmoles or 6 x 10^{12} molecules	100 µg
120,000	8.3 pmoles or 5 x 10^{12} molecules	120 µg
140,000	7.1 pmoles or 4.3 x 10^{12} molecules	140 µg
160,000	6.3 pmoles or 3.8 x 10^{12} molecules	160 µg
180,000	5.6 pmoles or 3.3 x 10^{12} molecules	180 µg
200,000	5 pmoles or 3 x 10^{12} molecules	200 µg

II. Some useful nucleotide dimensions

1 cm of DNA ~ 3 x 10^6 nucleotides

Organism	Base pairs/ haploid genome	Base pairs/ diploid genome	Length/cell	Mass

Human	3 x 10^9	6 x 10^9	2 meters (diploid)	6 pg
Fly	1.65 x 10^8	3.3 x 10^8	100 cm (diploid)	0.3 pg
Yeast	1.35 x 10^7	2.7 x 10^7	10 cm (doiploid)	0.03 pg
E. coli	4.7 x 10^6	-	1.5 cm (diploid)	0.0045 pg
SV40	5 x 10^3	-	1.7 nm	0.000006 pg

III. Some useful cell dimensions

Organism	**Dimensions**	**Volume**
S. cerevisiae	5 µm	66 µm^3
S. pombe	2 x 7 µm	22 µm^3
Mammalian cell	10-20 µm	500-4,000 µm^3
E. coli	1 x 3 µm	2 µm^3
Mammalian mitochondrion	1 µm	0.5 µm^3
Mammalian nucleus	5-10 µm	66-500 µm^3
Plant chloroplast	1 x 4 µm	3 µm^3
Bacteriophage lambda	50 nm (head only)	6.6 x 10^{-5} µm^3
Ribosome	30 nm diameter	1.4 x 10^{-5} µm^3
Globular monomeric protein	5 nm diameter	6.6 x 10^{-8} µm^3

III. Some useful concentrations

<u>Total cell protein concentration</u>	Detergent soluble protein = 1-2 mg/ 10^7 mammalian cells or 100-200 mg/ ml for soluble proteins only
<u>Specific protein concentrations</u>	
Nucleus (200 µm^3):	
Abundant transcription factor	1 nM (100,000 copies/ nucleus)
Rare transcription factor	10 pM (1,000 copies/ nucleus)
<u>Serum</u>	50-100 mg/ ml

IV. Some useful Conversiontables

<u>**Molar conversions for protein**</u>

100 pmol	**µg**
10,000 Da protein	1

100,000 Da protein	10

Protein/ DNA conversions

1 kb of DNA encodes 333 amino acids ~/= 3.7 x 10^4 Da

Protein	DNA
10,000 Da	270 dp
30,000 Da	810 dp
100,000 Da	2,7 dp

Nucleic acid content of a typical human cell

DNA per cell	~ 6 pg
Total RNA per cell	~ 10-30 pg
Proportion of total RNA in nucelus	~ 14%
DNA:RNA in nucleus	~2:1
Human genome size (haploid)	3.3 x 10^9 bp
Coding sequences/ genomic DNA	3%
Number of genes	0.5-1 x 10^5
Active genes	1.5 x 10^4
mRNA molecules	2 x 10^5 - 1 x 10^6
Typical mRNA size	1900 nt

RNA distribution in a typical mammalian cell

RNA species	Relative amount
rRNA (28S, 18S, 5S)	80-85%
tRNAs, snRNAs, low MW species	15-20%
mRNAs	1-5%

RNA content in various cells and tissues

Source		Total RNA	mRNA (µg)
Cell cultures (10^7 cells)		30-500	0.3-25
	NIH/3T3	120	3
	HeLa	150	3
	COS-7	350	5
Mouse-developmental stages (per organism)	Unfertilized egg	0.43 ng	nd
	Oocyte	0.35 ng	nd

	2-cell	0.24 ng	nd
	8-16-cell	0.69 ng	nd
	32-cell	1.47 ng	nd
	13-day-old-embryo	450	13
Mouse tissue (100 mg)	Brain	120	5
	Heart	120	6
	Intestine	150	2
	Kidney	350	9
	Liver	400	14
	Lung	130	6
	Spleen	350	7

nd = not determined

Human blood*: cell, DNA, RNA, and protein content

	Leukocyes	**Thrombocytes**	**Erythrocytes**
Function	Immune response	Wound closing	O_2 & CO_2 transport
Cells per ml	4-7 x 10^6	3-4 x 10^8	5 x 10^9
DNA content	30-60 µg/ ml blood (6 pg/cell)		
RNa content	1-5 µg/ ml blood		
Hemoglobin content			~150 mg/ ml blood (30 pg/cell)
Plasma protein content		60-80 mg/ ml	

*From a healthy individual. The leukocyte concentration can vary from 2 x 106 per ml in cases of immunosuppression, to 40 x 10^6 during inflammation, to 500 x 10^6 during leukemia. The DNA and RNA content will vary accordingly.

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